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Histologic Analysis of the Thermal Effect on Epidermal and Dermal Structures Following Treatment With the Superpulsed CO_2 Laser and the Erbium:Yag Laser: An In Vivo Study

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Background and Objective: **To compare the in vivo histologic effects of the carbon dioxide (CO_2) and erbium:yttrium aluminum garnet (Er:YAG) lasers. To ascertain the effects of combining CO_2 and Er:YAG laser modalities during a single treatment session.**
Study Design/Materials and Methods: **Ten patients underwent laser treatment to four left preauricular sites 7 days prior to rhytidectomy as follows: CO_2 alone, CO_2/Er:YAG, Er:YAG alone, and Er:YAG/CO_2. The right preauricular area was identically treated 1 hour prior to rhytidectomy. Laser treated skin was excised during rhytidectomy and was evaluated histopathologically in a blinded manner.**
Results: **After 7 days, all groups were reepithelialized and showed equal neo-collagen formation. After 7 days, CO_2/Er:YAG and Er:YAG alone had the least collagen injury and thickest epidermis and papillary dermis of all groups. Specimens lased 1 hour prior to excision showed the least collagen injury and thermal necrosis when treated with CO_2/Er:YAG and Er:YAG alone. Four passes with CO_2 removed 250 μm of tissue, while eight passes with the Er:YAG removed 160 μm of tissue.**
Conclusions: **Limiting CO_2 laser passes and ending with Er:YAG produces less collagen injury, less thermal necrosis, and more robust epithelial and dermal fibrous tissue regeneration. CO_2 followed by Er:YAG has similar thermal necrosis and collagen injury as Er:YAG alone, presumably due to Er:YAG removal of CO_2 induced thermal injury. Lasers Surg. Med. 24:93–102, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: ablation; collagen; dermis; epidermis; facial; resurfacing; skin

INTRODUCTION

Rapid advances in the development of laser technologies utilized for skin resurfacing demand careful evaluation of the respective histopathologic effects of each new technology. While the pulsed CO_2 laser has been studied extensively with respect to histopathologic effect and clinical results

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[1–6], there is relatively less data available for the Er:YAG laser, a more recently applied technology for facial skin resurfacing.

The pulsed CO_2 laser is generally understood by laser skin resurfacing practitioners to have a beneficial collagen tightening effect, while having the potential for undesirable wound healing such as prolonged erythema and delayed reepithelialization. The Er:YAG laser, on the other hand, is generally considered to be less injurious to tissue than CO_2, while having potentially less collagen contraction effect and perhaps less dramatic skin tightening.

The objectives of this study:

1. Compare the histologic effects of the CO_2 and Er:YAG lasers in an in vivo human model based on findings of thermal necrosis, degree of reepithelialization, skin layer thickness, and new collagen formation.
2. Determine the tissue effects of combining CO_2 and Er:YAG laser treatments at the same tissue site during the same treatment session by evaluating collagen injury, thermal necrosis, and ablation depth.
3. Identify an ideal laser combination to exploit the most favorable tissue effects of the CO_2 and Er:YAG lasers, while minimizing the less desirable side effects of each laser.

MATERIALS AND METHODS

Ten consecutive patients scheduled to undergo cervicofacial rhytidectomy were offered enrollment in this Stanford University Human Subjects in Medical Research approved laser study. All patients accepted the opportunity to enroll in this protocol.

Two lasers were utilized. The Luxar LX-20SP Novapulse™ CO_2 laser (10,600 nm) with SureScan Computer Pattern Generator (CPG) (Luxar Corporation, Bothell, Washington) was used in the superpulse mode at 6 Watts, 16 Hertz (Hz), and a 730 μsec pulse duration. The 7.6 mm by 6.4 mm CPG parallelogram was selected. This laser delivered a fluence of 4.7 J/cm^2. The ESC Derma-20™ Er:YAG laser (2,940 nm)(ESC Medical Systems, Yokneam, Israel) was used with a 6 mm spot size at 14 W and 8 Hz, with a 350 μsec pulse duration, to deliver 1.7 J/pulse and a fluence of 4.7 J/cm^2. These parameters were selected to provide identical fluences between the laser systems compared in this study.

TABLE 1. Grading Scale (Gross Skin Abnormalities and Histopathologic Evaluation)

Grade	Degree of abnormality
0	0%, no findings, normal
1	20%, mild abnormality
2	40%, mild-moderate abnormality
3	60%, moderate abnormality
4	80%, moderate-severe abnormality
5	100%, severe abnormality

Fitzpatrick skin types [7] were determined for each patient. The degree of clinically evident facial skin actinic damage and rhytidosis was graded preoperatively for each patient using a 5-point scale (Table 1).

Seven days prior to rhytidectomy, each patient underwent injection of 2 cc of 2% lidocaine in the subcutaneous plane of the left preauricular region, avoiding dermal infiltration. Single or combination laser energy was applied at 4 separate target sites as follows: CO_2 (four passes), CO_2 (two passes) followed by Er:YAG (four passes), Er:YAG (eight passes), Er:YAG (four passes) followed by CO_2 (two passes). All laser treatments were performed by the same operator (DSU) using identical techniques. Wiping of the treated skin was performed after each pass with the CO_2 laser but not after each pass with the Er:YAG laser. This was done to reflect typical clinical practice.

One week later, 1 hour prior to rhytidectomy, the right preauricular region was treated in an identical manner at four target sites (Fig. 1A,B). A standard rhytidectomy was then performed with immediate excision of the laser treated skin (Fig. 2). Tissue trauma was minimized to reduce potential edema formation. After excision, each laser site was identified, separated from adjacent skin, and placed in an individually labeled container in 10% formalin. An additional untreated specimen was submitted similarly as control. The rhytidectomy procedure was completed in the usual manner.

One histopathology technician prepared all 90 specimens on the same day. Hematoxylin and eosin (H and E) staining was utilized. Each slide was labeled with a study code allowing later identification of each specimen as to the type of laser used and the time interval of treatment. The study dermatopathologist (BME) remained blinded to this code throughout the histologic evaluation process. The code was revealed after

Fig. 1. **(A)** Left preauricular region treated with laser 7 days previously at 4 sites. Lesions are well healed. **(B)** Same patient, right preauricular region immediately after treatment of four sites in the identical manner. From superior to inferior: CO2 alone; CO2 followed by Er:YAG (superior-most round lesion); Er:YAG alone; Er:YAG followed by CO2.

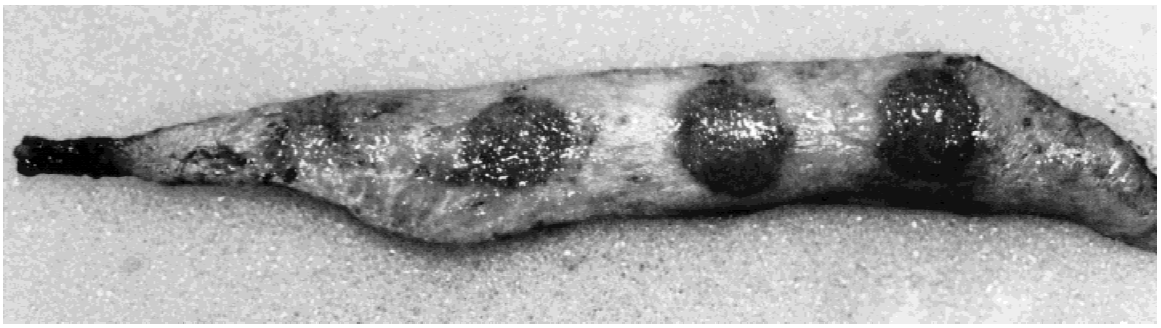

Fig. 2. Skin excised during rhytidectomy with four laser treatment sites evident.

all histopathologic data was recorded and ready for analysis.

Control specimens were histologically graded using a 5 point scale (Table 1) for degree of melanocyte atypia, hypertrophy, and hyperplasia, as well as epidermal atypia, polarity abnormalities, and parakeratosis. Dermal elastosis was graded. An optical micrometer was used to determine the thickness (μm) of the epidermis, papillary dermis, and reticular dermis.

Specimens treated with the laser 7 days prior to excision were graded for degree of reepithelialization, new collagen formation, collagen injury, and inflammation. An optical micrometer was used to determine the thickness (μm) of the epidermis, papillary dermis, and reticular dermis.

Specimens treated 1 hour prior to excision were graded for irregularity of ablation, collagen injury, and inflammation. The optical micrometer was used to determine the thickness (μm) of the thermal necrotic zone and the distance from the ablated surface to the dermal-subcutaneous junction.

The Student's *t*-test was utilized to determine the significance of differences detected between groups. Differences at the 5% level were considered statistically significant.

RESULTS

Ten patients [seven male, three female, mean (± SD) age 49.7 ± 6.6 years] were enrolled as candidates for rhytidectomy and participation in this laser study. All were Fitzpatrick skin types II or III, mean 2.3 ± 0.5. Gross actinic skin damaged ranged from 1 to 4, mean 2.5 ± 1.0. Degree of facial rhytidosis ranged from 1 to 4, mean 2.2 ± 0.8. Control specimens demonstrated mild-moderate melanocyte atypia and hypertrophy, but minimal hyperplasia (Table 2). There was mild-moderate epidermal atypia and polarity abnormalities, minimal parakeratosis, and marked dermal elastosis. Values for mean control skin layer thickness are shown in Table 2.

TABLE 2. Control Skin Specimens (Untreated)*

Histologic finding	Grade (mean ± SD)
Melanocytye atypia	1.4 ± 0.8
Melanocytye hypertrophy	1.5 ± 0.8
Melanocytye hyperplasia	0.2 ± 0.4
Epidermal atypia	2.0 ± 0.9
Polarity abnormalities	1.9 ± 0.9
Parakeratosis	0.6 ± 0.5
Dermal elastosis	3.6 ± 1.0
Skin layer	Thickness (mean ± SD)
Epidermis	64.5 ± 19 μm
Epidermis	7.5 ± 1.6 cells
Papillary dermis	55 ± 28 μm
Reticular dermis	1,360 ± 330 μm

*The scale utilized for grading these histopathologic findings is described in Table 1.

All specimens were re-epithelialized after 7 days (Fig. 3) (Table 3). New collagen was seen in all groups after 7 days (Fig. 4) with no statistically significant difference between groups. There was less evidence of collagen injury in the CO_2/Er:YAG and Er:YAG alone groups, than the CO_2 alone and Er:YAG/CO_2 groups; however these differences did not reach statistical significance. There was no statistically significant difference in the amount of inflammation detected between groups after 7 days.

Epidermal thickness after 7 days was greater than controls in all groups ($P<0.003$ for all groups) (Table 4). Epidermal thickness was greatest in the Er:YAG alone group (Fig. 5) and least in the CO_2 alone group. There was a statistically significant difference when comparing Er:YAG alone and Er:YAG/CO_2 to CO_2 alone ($P=0.005$ and $P=0.0001$, respectively). Papillary dermal thickness after 7 days was greater than controls in all groups ($P=.053$ for CO_2 alone group,

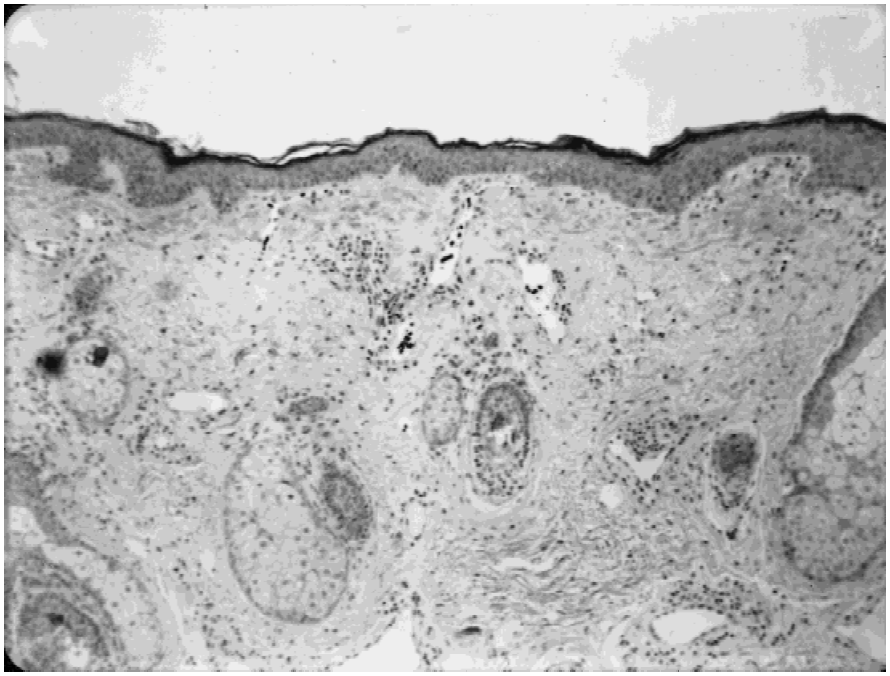

Fig. 3. Photomicrograph of a specimen treated with the CO2 laser 7 days prior to excision. Note complete reepithelialization. (×70, H and E stain).

TABLE 3. Histologic Evaluation (Laser Treatment 7 Days Prior to Excision)*

	Reepithelialization	Neo-collagen	Collagen Injury	Inflammation
CO_2 alone	5.0 ± 0	1.9 ± 1.4	0.8 ± 0.9	1.8 ± 0.4
CO_2/Er:YAG	5.0 ± 0	1.7 ± 1.3	0.4 ± 0.5	2.1 ± 0.3
Er:YAG alone	5.0 ± 0	1.9 ± 1.2	0.3 ± 0.5	2.0 ± 0
Er:YAG/CO_2	4.9 ± 0.3	1.8 ± 1.0	0.6 ± 0.5	2.1 ± 0.6

*The scale utilized for grading these histopathologic findings is described in Table 1.

$P = <0.002$ for remaining groups). Papillary thickness was greatest in the Er:YAG alone group and least in the CO_2 alone group. There was a statistically significant difference when comparing Er:YAG alone to CO_2 alone ($P = 0.02$). Reticular dermis thickness after 7 days was less than controls in all groups, but this difference was not statistically significant. There was no statistically significant difference in reticular dermis thickness between groups.

Specimens excised 1 hour after laser treatment were uniformly ablated (Fig. 6) (Table 5). There was less evidence of thermal collagen injury in the CO_2/Er:YAG and Er:YAG alone groups, compared with the CO_2 alone and Er:YAG/CO_2 groups (Fig. 7). These observed differences were statistically significant in three of four comparisons as follows: CO_2:Er:YAG vs. CO_2 alone and Er:YAG/CO_2 ($P = 0.047$ and $P = 0.0004$, respectively); Er:YAG alone vs. CO_2 alone and Er:YAG/CO_2 ($P = 0.08$ and $P = 0.002$, respectively).

There was less thermal necrosis in the CO_2/Er:YAG and Er:YAG alone groups, compared with the CO_2 alone and Er:YAG/CO_2 groups (Fig. 8). These observed differences were statistically significant: CO_2:Er:YAG versus CO_2 alone and Er:YAG/CO_2 ($P = 0.007$ and $P = 0.003$, respectively); Er:YAG alone versus CO_2 alone and Er:YAG/CO_2 ($P = 0.007$ and $P = 0.003$, respectively).

Inflammation after 1 hour was least in the CO_2/Er:YAG group and greatest in the CO_2 alone group (Table 5). In the CO_2 alone group, four passes with this laser resulted in a mean ablation (reduction in total skin thickness) of 250 ± 540 μm (mean 62.5 μm per pass). In the Er:YAG alone group, eight passes with this laser resulted in a mean ablation of 160 ± 528 μm (mean 20 μm per pass).

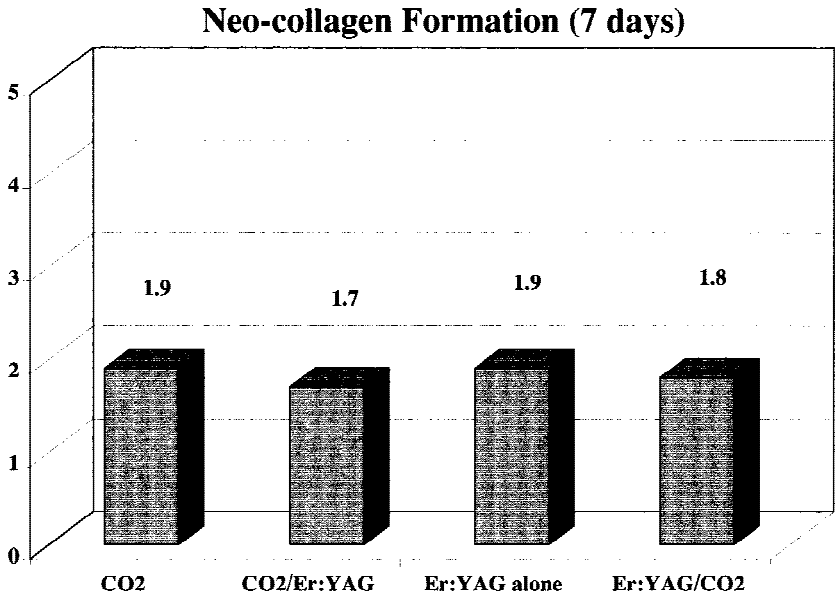

Fig. 4. Neo-collagen formation after 7 days comparing all four laser groups. No statistically significant difference exists between groups at this time point.

TABLE 4. Skin Thickness (Laser Treatment 7 Days Prior to Excision)

	Epidermis (μm)	Papillary dermis (μm)	Reticular dermis (μm)
Control	64.5 ± 19	55 ± 28	1,360 ± 330
CO_2 alone	88 ± 21	87 ± 76	1,195 ± 501
CO_2/Er:YAG	112 ± 37	128 ± 60	1,175 ± 312
Er:YAG alone	163 ± 67	151 ± 77	1,140 ± 255
Er:YAG/CO_2	155 ± 48	135 ± 62	1,230 ± 250

DISCUSSION

The CO_2 laser has an established record of safety and efficacy for the application of facial skin resurfacing [1,2,6,8]. The advantages of the CO_2 laser are reported as thermal contraction of collagen [4,9], operator familiarity with existing technology, improved technology with addition of CPG handpiece [4,10], relatively lower equipment costs than alternative lasers, and adequate delineation by previous authors of the histologic tissue effects and clinical outcomes expected from this laser [1,2,3,8,11,12]. Potential disadvantages of the CO_2 laser include the creation of a thermal injury zone deep to the ablation zone [1,2,3,12], as well as a diminishing ablation depth and increasing thermal necrosis zone with each successive pass [13,14]. Excessive thermal injury has been implicated as a possible etiology for prolonged erythema, hyperpigmentation, and delayed wound healing sometimes observed after treatment with the CO_2 laser [15,16]. Er:YAG has a ten-times higher coefficient of absorption in water (7,700 cm^{-1}) than CO_2 and a reduced optical penetration in tissue (1 μm vs 20–30 μm) [17,18]. The Er:YAG laser is reported to have less thermal necrosis and less tissue ablation per pass and therefore less erythema and wound healing complications [17,19,20].

Much of the early data for laser histologic tissue effect has been derived from ex vivo treatment of skin [4,14,21], although more recently published reports utilize in vivo models (human and animal) [1–3]. To determine the tissue effects of the CO_2 and Er:YAG laser in the most clinically applicable setting, an in vivo human skin model was utilized. Sundamaged, preauricular skin was selected to provide the closest semblance to a facial skin resurfacing scenario. The degree of dermal elastotic material seen in the control specimens is indicative of this exposed, sundamaged area. In addition to evaluating the tissue effects of each laser individually, combination laser treatment (i.e., CO_2 followed by Er:YAG) was evaluated. The number of passes and power settings for each laser in this protocol were selected to approximate actual resurfacing practices and to provide an identical fluence per pass. The authors recognize that 2–3 passes with the CO_2 laser may be the usual number performed to achieve clinically desirable results. We chose four passes with the CO_2 in order to guarantee a detectable repeatable histologic result and to have the ability to

Fig. 5. Photomicrograph of a specimen treated with Er:YAG laser alone 7 days prior to excision. Note thickened epidermis during this reparative phase. (×90, H and E stain)

Fig. 6. Photomicrograph of a specimen treated 1 hour prior to excision with CO_2 laser followed by Er:YAG laser. Note uniform ablation surface, regular thermal necrotic zone, and no retained epithelium. (×90, H and E stain)

halve the number of CO_2 laser passes for subsequent combination laser groups.

All specimens were reepithelialized after 7 days. There may be groups that healed fully prior to 7 days, but this study was not designed to detect these healing differences. New collagen was seen equally in all groups after 7 days. Longer follow-up is required to determine if a difference

TABLE 5. Histologic Evaluation (Laser Treatment 1 Hour Prior to Excision)

	Irregularity of ablation	Collagen injury	Inflammation	Thermal necrosis (μm)	Ablation thickness (μm)
CO_2 alone	0.3 ± 0.5	1.3 ± 0.5	1.8 ± 0.6	89 ± 38	250 ± 540
CO_2/Er:YAG	0.2 ± 0.6	0.8 ± 0.6	1.2 ± 0.4	56 ± 22	260 ± 487
Er:YAG alone	0.3 ± 0.7	0.9 ± 0.6	1.4 ± 0.5	43 ± 19	160 ± 528
Er:YAG/CO_2	0.2 ± 0.6	1.7 ± 0.5	1.5 ± 0.5	97 ± 36	130 ± 457

The scale utilized for grading these histopathologic findings is described in Table 1.

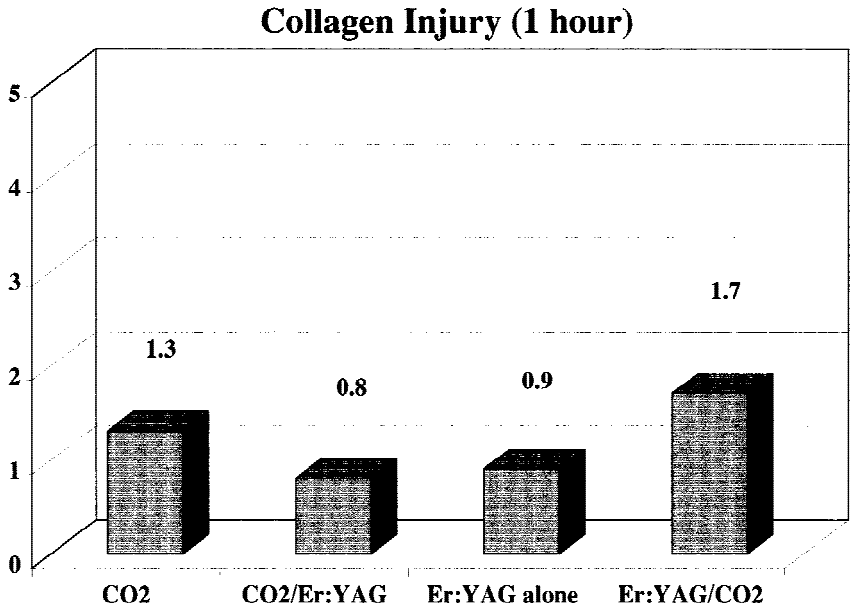

Fig. 7. Bar graph illustrating degree of collagen injury of each group treated with laser 1 hour prior to excision. Specimens treated with CO_2/Er:YAG or Er:YAG alone demonstrated the least amount of collagen injury.

would develop between groups. Although the differences were not statistically significant, the CO_2/Er:YAG and Er:YAG alone groups had the least collagen injury suggesting: Er:YAG following CO_2 removes the CO_2 induced thermal injury and; Er:YAG alone causes less thermal injury.

Epidermal and papillary dermal thickness was greater than control after 7 days in all groups. This is indicative of the reparative phase of healing [3]. There was, however, a greater epidermal and papillary dermal thickness in the CO_2/Er:YAG, Er:YAG alone, and Er:YAG/ CO_2 groups when compared to CO_2 alone. Fewer passes with the CO_2 laser and less resultant thermal injury by ending with Er:YAG may allow more exuberant reepithelialization and dermal regeneration.

In the specimens excised 1 hour after laser treatment, CO_2/Er:YAG and Er:YAG alone had the least amount of thermal necrosis and collagen injury. These findings parallel those of the 7 day group indicating, again, that Er:YAG following CO_2 removes the CO_2 induced zone of thermal necrosis and that Er:YAG alone causes less thermal injury. Previous authors report that a single pass with the CO_2 laser at typical settings ablates 50–150 μm of tissue [2,4,14,21,22,23], while creating a zone of thermal necrosis of up to 100 μm [3,4,14,21]. This study found that four passes with CO_2 ablated a mean tissue thickness of 250 ± 540 μm (62.5 μm per pass). The CO_2 laser may ablate less tissue thickness and create additional thermal necrosis with each successive pass [13,14], but this could not be ascertained in this study. Previous authors have reported that a single pass with the Er:YAG laser at typical settings ablates between 10–40 μm [20,24], while creating less thermal necrosis (0–50 μm) [19,25,26]. This study found that eight passes with the Er:YAG ablated a mean tissue thickness of 160 ± 528 μm (20 μm per pass).

While the mean ablation depths from this

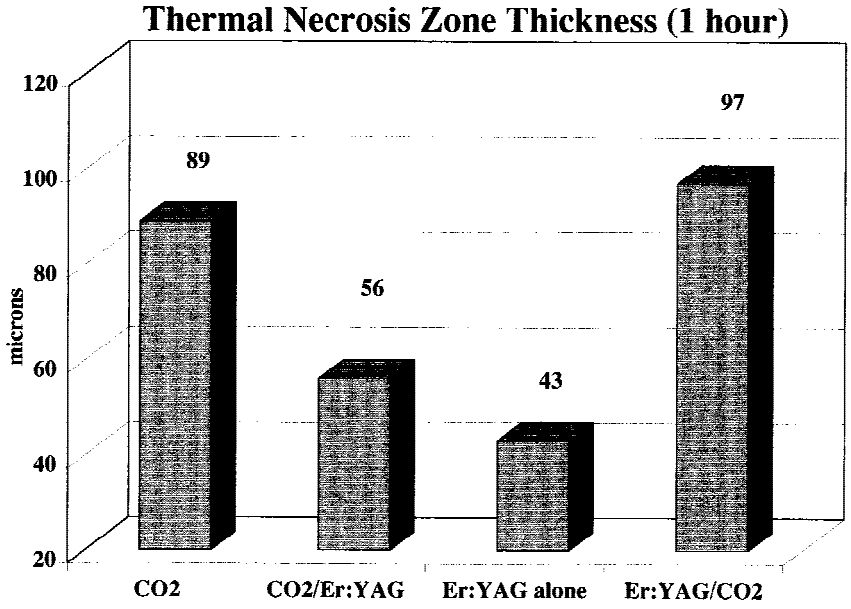

Fig. 8. Bar graph illustrating thickness of the thermal zone of necrosis (in μm) of each group treated with laser 1 hour prior to excision. Specimens treated with CO_2/Er:YAG or Er:YAG alone demonstrated the least amount of thermal necrosis.

study compare favorably with previous author's data, there was variation in the amount of ablation detected for each specimen. Evaluating ablation depth requires comparison of pre and post-treatment skin measured with an optical micrometer from the normal or ablated surface to the dermal-subcutaneous junction. Skin thickness and dermal-subcutaneous junction depth vary even within a single small specimen; therefore several measurements were taken from each specimen. Tissue edema, injection injury, surgical manipulation injury, and histopathologic embedding trauma all further contribute to the difficulty inherent to making precise, reliable measurements. The difficulty in determining ablation depth was reported as well by Fitzpatrick et al. [14]. The reader should interpret the ablation depths per pass reported in this study as average, approximate values, and should be aware of the difficulties inherent to determining ablation depth when evaluating other reports on laser resurfacing.

A limitation of this study is that follow-up is limited to 7 days. It is possible that a long term difference in the amount of neo-collagen between laser groups exists, and that this difference is not detectable at 7 days. A study is currently underway to evaluate the histological findings of these laser combinations after a 3 month interval. A second limitation is that these patients did not undergo full face resurfacing; therefore clinical correlation with the histologic findings is not possible. Finally, each laser or combination of lasers may have varying tissue edema producing effects, which could confound the determination of accurate ablation and thermal injury depths.

Our clinical experience reflects the specific histologic findings of this study. Our patients who undergo CO_2 laser resurfacing typically require 7 days to completely heal and may have erythema for up to 3 months. Despite these apparent disadvantages, CO_2 laser patients have very satisfying results in terms of skin tightening, skin rejuvenation, and removal of rhytids. The thermal injury imparted by the CO_2 laser may be implicated as causing both the prolonged healing and improved results. Er:YAG patients, on the other hand, tend to heal in 3–4 days, have less erythema, and have fewer wound complications, yet may have somewhat less satisfying rhytid resolution and skin tightening.

The results of this study have prompted us to begin treating patients with combination laser therapy (two passes with the CO_2 laser followed by 2–4 passes with the Er:YAG laser) in order to exploit the specific advantages of each laser. Wound healing with this combination treatment is expedient, usually within 4 days (like Er:YAG alone), while patient satisfaction with skin rejuvenation, rhytid removal and tightening are high (like CO_2 alone). Perhaps the rate and vigor of the healing process is related to the amount of re-

sidual thermal necrosis as opposed to the depth of ablation achieved. We will continue to treat patients in this manner, while we initiate a long-term follow-up study evaluating the histologic effects of this laser combination.

CONCLUSIONS

1. Reepithelialization in this series occurred in all groups by 7 days.
2. New collagen formation was observed equally in all groups at 7 days.
3. CO_2 followed by Er:YAG specimens and Er:YAG alone specimens had the least amount of collagen injury and thermal necrosis.
4. Minimizing the number of passes with CO_2 and ending with Er:YAG may allow more robust reepithelialization and dermal regeneration.
5. Specimens treated with CO_2 followed Er:YAG has similar amounts of collagen injury and thermal necrosis as Er:YAG alone. This is likely due to partial removal of the CO_2 laser induced thermal necrosis with the Er:YAG laser as the final step in the treatment. This is the treatment regimen we currently utilize for our laser skin resurfacing patients.

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